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TITLE: Non-Histaminergic Itch Mediators Elevated in the Skin of a Porcine Model of Scabies and of Human Scabies Patients

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SHORT TITLE: Itch mediators elevated in scabies skin

ABBREVIATIONS USED: TRPV1, transient receptor potential vanilloid 1; TRPA1, transient receptor potential ankyrin 1, PAR-2, protease-activated receptor 2; ENFD, epidermal nerve fiber density

LETTER

To the Editor:

Scabies, caused by infestation of the *Sarcoptes scabiei* mite, has been estimated to have a prevalence of 146 million people worldwide (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017). A hallmark of scabies is intense itch that worsens at night (Jannic et al., 2018). In a study of children and adults with scabies, 94.5% of patients reported pruritus (Boralevi et al., 2014). A questionnaire study found an average itch intensity of 7.2 out of 10 on the visual analogue scale for scabies patients (Shin et al., 2017). In another study, 36.3% of patients described their itch as “intense” or “severe” (Worth et al., 2012). Itch can continue for up to four weeks after scabies treatment (Chosidow, 2006) and has important consequences for quality of life (Shin et al., 2017, Worth et al., 2012). Additionally, scratching can lead to secondary bacterial infection of the skin (impetigo), which can have life-threatening complications (Heukelbach and Feldmeier, 2006). However, the molecular mechanisms of scabies itch are largely unknown.

Though histamine is considered the classical pruritogen, most types of chronic itch fail to respond to antihistamine treatment. Recent studies have emphasized the importance of nonhistaminergic and neural mechanisms in chronic itch conditions (Sanders et al., 2016). The ion channels transient receptor potential (TRP) vanilloid 1 (TRPV1) and TRP ankyrin 1 (TRPA1) are expressed on itch-signaling primary afferent neurons. Tryptase, expressed by mast cells, and its receptor protease-activated receptor 2 (PAR-2) are major nonhistaminergic itch mediators in diseases like atopic dermatitis. We hypothesized that these mediators may also play a role in scabies-related itch. Additionally, numerous dermatoses characterized by itch are known to alter epidermal innervation, and such changes may underlie persistent itch following

treatment. For this reason, we examined whether epidermal nerve fiber density (ENFD) is altered in scabies. An increase in ENFD would indicate that scabies induces epidermal hyperinnervation.

To investigate the potential mechanisms of scabies itch, we collected samples from scabies-infested and healthy control skin of pigs and humans. The porcine model of scabies was induced as previously described (Bernigaud et al., 2016, Mounsey et al., 2010); see Supplementary Materials. Three female *Sus scrofa domesticus* “large white” pigs were used for the experiment. Mite-infested skin crusts were directly transplanted into the ear canal of the naive pigs in order to induce a scabies infestation. Biopsies were taken from areas of lesional, mite-infested skin and non-lesional (clinically normal in appearance), mite-free skin along the back and neck.

Human samples included de-identified skin biopsies from 6 scabies patients (3 male/3 female, age 65.00 ± 4.58) and 4 healthy control volunteers (2 male/2 female, age 63.33 ± 10.21). Samples were obtained from the University of Miami Department of Dermatology & Cutaneous Surgery. Biopsies were taken from the abdomen. For scabies patients, diagnosis was confirmed by visualization of mites via microscopy of lesional skin adjacent to the tissue used for this study. Use of de-identified samples was considered non-human research by the University of Miami Institutional Review Board and did not require patient consent.

Using immunohistochemistry, we quantified the expression of the itch mediators TRPV1, TRPA1, PAR-2, tryptase, and histamine in both pig and human samples (see Supplementary Materials). We also quantified expression of β -tubulin in order to identify changes in ENFD.

In the porcine model (Figure 1), epidermal expression of TRPV1, TRPA1, and PAR-2 was increased in tissue infested with scabies ($p=0.031$, $p<0.0001$, and $p=0.002$, respectively). The number of tryptase+ cells near the dermal-epidermal junction was increased in porcine tissue

infested with scabies ($p=0.021$). The number of histamine+ cells did not differ between scabies-infested and control skin ($p=0.73$). ENFD did not differ between scabies and control tissue ($p=0.059$).

In human samples (Figure 2), epidermal expression of TRPV1, TRPA1, and PAR-2 was elevated in scabies-infested tissue compared to healthy control ($p=0.002$, $p=0.027$, and $p=0.010$, respectively). Furthermore, the number of tryptase+ cells near the dermal-epidermal junction was increased in human tissue infested with scabies ($p=0.043$). However, the number of histamine+ cells near the dermal-epidermal junction was decreased in scabies-infested tissue ($p=0.003$). ENFD was elevated in human skin infested with scabies ($p=0.037$).

In conclusion, we found significant elevations of nonhistaminergic itch mediators in the skin of a pig model experimentally infested with scabies and of human scabies patients. In both species, scabies infestation induced statistically significant elevations in expression of TRPV1, TRPA1, and PAR-2 in the epidermis and in the number of tryptase+ cells near the dermal-epidermal junction. For these results, the pig model showed remarkable similarities to the human patients with scabies, suggesting that this model is well suited to study scabies-related itch. The slight differences between the pig and human samples (number of histamine+ cells and ENFD) may be explained by several possible factors, such as biopsy site, duration of scabies, scratching of the skin, or general species differences. Future work will be needed to confirm these results in a larger sample size.

Though antihistamines have been suggested as an adjunct therapy to treat scabies itch (Hengge et al., 2006, Mounsey and McCarthy, 2013, Salavastru et al., 2017), our results suggest that antihistamine treatment is unlikely to reduce scabies itch. The nonhistaminergic mediators

we identified are commonly overexpressed in other chronic itch diseases and may provide targets for treatment of this severe and bothersome symptom.

CONFLICT OF INTEREST

KMS, LAN, JDR, HHA, JH, PR, JG, and OC state no conflict of interest. CB received research support from Bioderma Laboratoire Dermatologique and Codexial Dermatologie to assist with the establishment and development of the scabies experimental porcine model in France. GY is a consultant and member of advisory board of Menlo, TREVI, Sienna, Galderma, Novartis, Sanofi, Pfizer and is funded by Sun Pharma and Pfizer.

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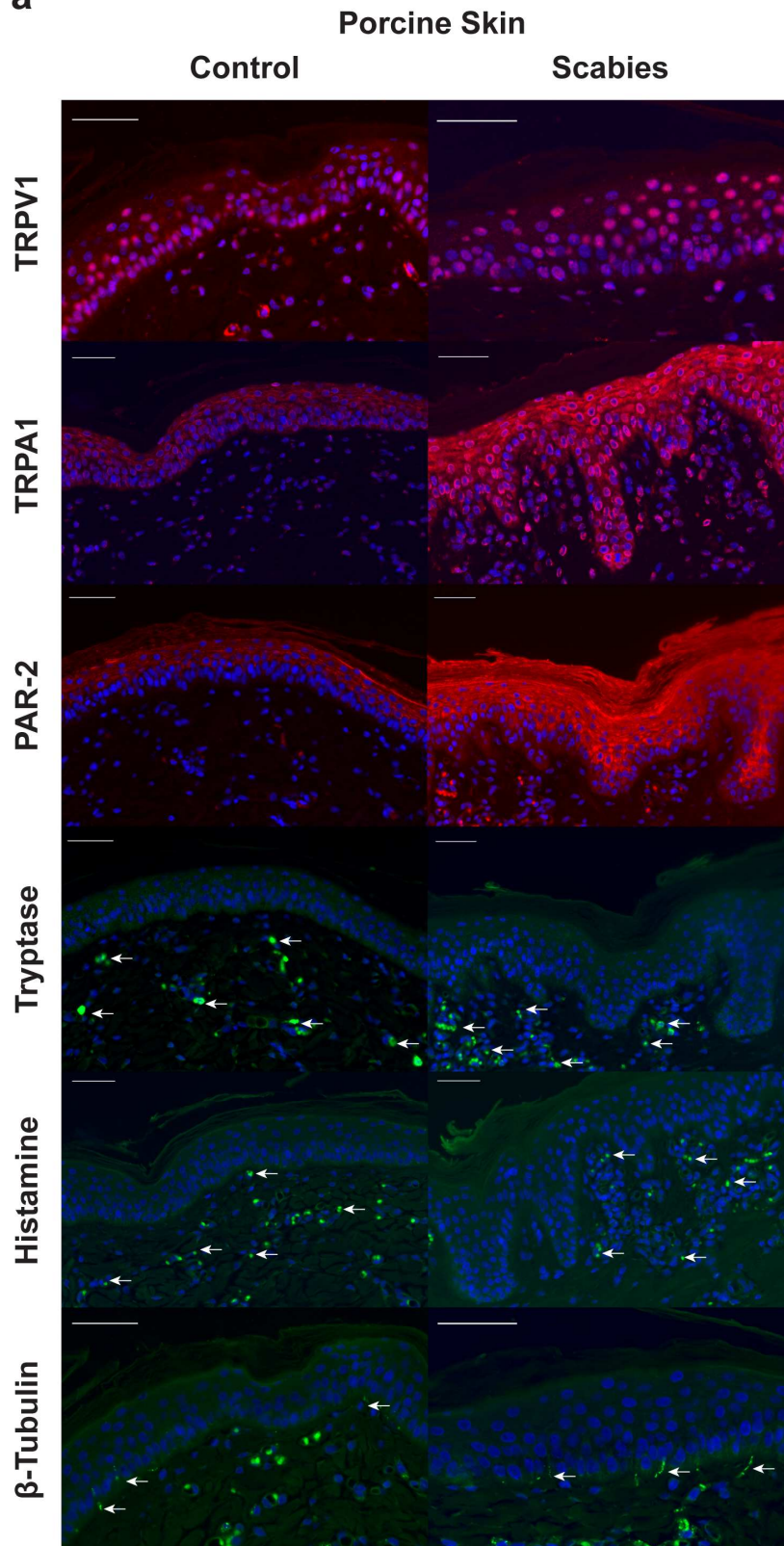
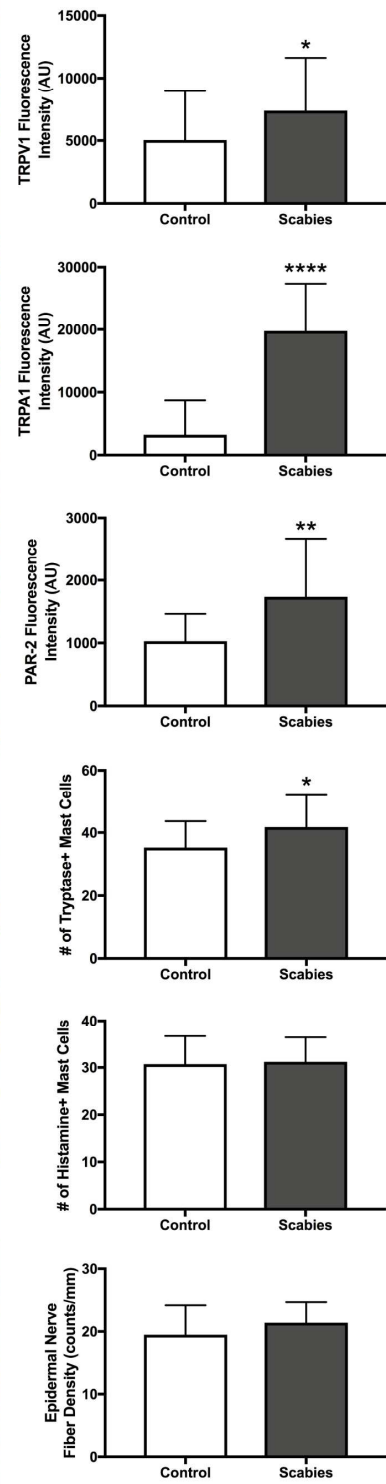
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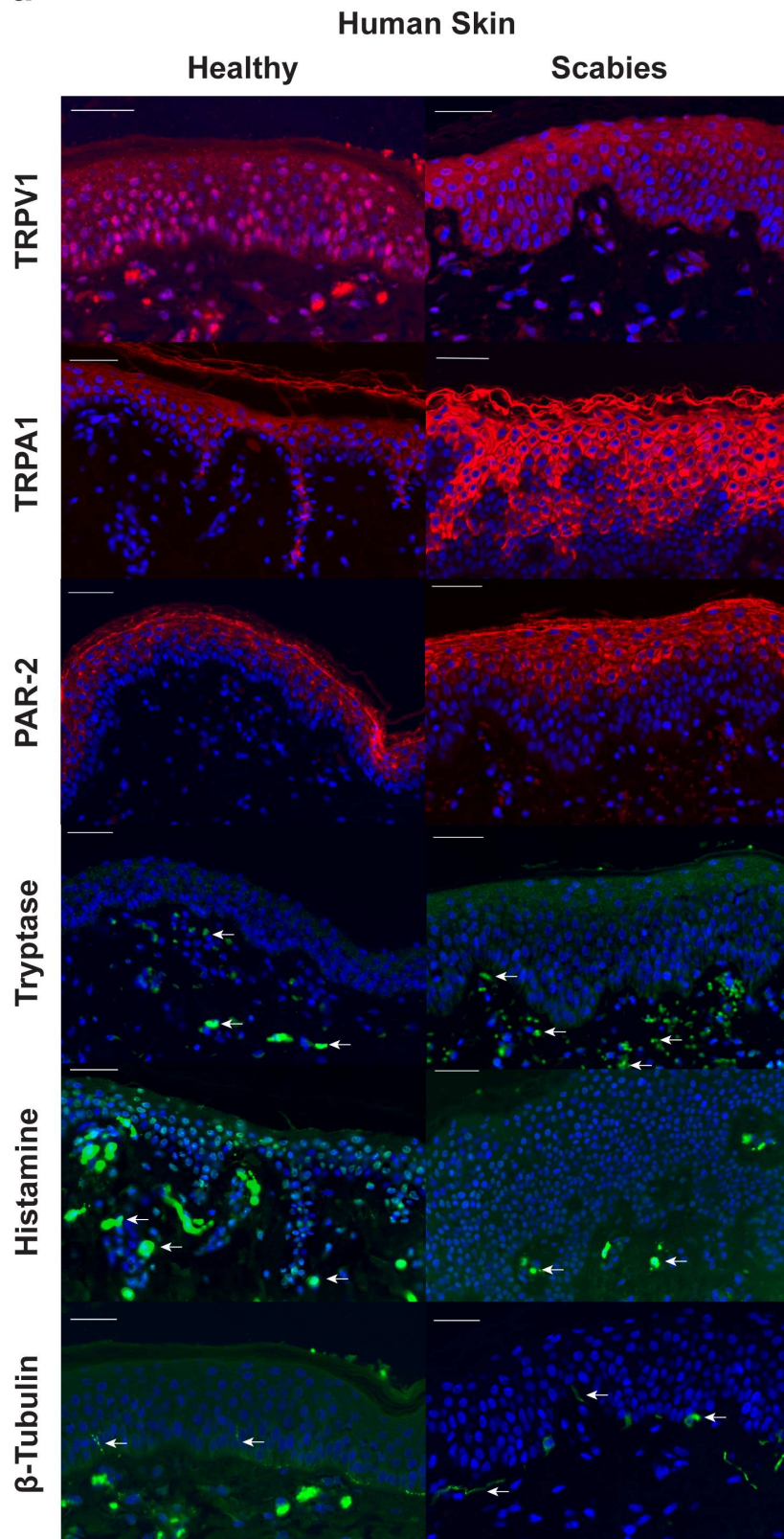
FIGURE LEGENDS

Figure 1. Non-histaminergic itch mediators were elevated in porcine skin with scabies. (A) Representative images of control (left) or scabies-infested (right) porcine skin immunostained for TRPV1, TRPA1, PAR-2, tryptase, histamine, and β -tubulin (n=3). Scale bar = 50 μ m. Arrows indicate examples of positively stained cells. (B) Quantification of immunostaining. Epidermal expression of TRPV1, TRPA1, and PAR-2 was increased in tissue infested with scabies. The number of tryptase+ cells was increased in porcine tissue infested with scabies. The number of histamine+ cells did not differ between scabies-infested and control skin. ENFD, visualized by β -tubulin staining, did not differ between scabies and control tissue. * = $p < 0.05$, ** = $p < 0.01$, **** = $p < 0.001$, unpaired t-test scabies vs control. AU = arbitrary units.

Figure 2. Non-histaminergic itch mediators were elevated in the skin of human patients with scabies. (A) Representative images of healthy (left) or scabies-infested (right) human skin immunostained for TRPV1, TRPA1, PAR-2, tryptase, histamine, and β -tubulin (n=4-6). Scale bar = 50 μ m. Arrows indicate examples of positively stained cells. (B) Quantification of immunostaining. Epidermal expression of TRPV1, TRPA1, and PAR-2 was elevated in scabies-infested tissue compared to healthy control. The number of tryptase+ cells was increased in human tissue infested with scabies. The number of histamine+ cells was decreased in scabies-infested tissue. ENFD, visualized by β -tubulin staining, was elevated in human skin infested with scabies. * = $p < 0.05$, ** = $p < 0.01$, unpaired t-test scabies vs control. AU = arbitrary units.

a**b**

a



b

